provide the first identification and characterization of the biophysical properties of the most prominently observed ion channel expressed within the inner and outer membrane of nuclei from adult skeletal muscle fibers. Excised inside-out single channel recordings were obtained from individual nuclei acutely isolated from Flexor Digitorum Brevis (FDB) fibers of wild-type mice. The outer membrane of nuclei was readily accessible following isolation. For measurements of channels from the inner membrane, nuclei were treated with 1 % (w/v) sodium citrate in order to remove the outer membrane. We found that the predominant ion channel expressed in both the inner and outer nuclear membrane was a cationic channel that conducts monovalent ions with slight preference for potassium over sodium ions (a PK/PNa ~1.22). A 10,000-fold difference in the concentration of free Ca2+ between the pipette and bath solutions did not affect the channel reversal potential in symmetric KCl (~0 mV), indicating that Ca2+ ions permeation is negligible. The maximum conductance of the channel in the outward direction was ~162 pS. The mean open probability (PO) was ~0.7 and voltage-independent between −50 mV to +50 mV. We suggest that this novel monovalent cationic channel within the inner and outer membrane of skeletal muscle nuclei provides a counter-current mechanism that minimizes voltage change across the nuclear membrane. This research was supported by NIH K01 award AR060831(to V.Y.) and NIH R01 grant AR44657 (to R.T.D).

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Novel Na^+-Selective Channels in the Lysosome

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Lysosomes, traditionally believed to be the terminal ''recycle center'' for biological ''garbage'', are now known to play indispensable roles in membrane trafficking and intracellular signaling pathways. The multiple functions require the establishment of lysosomal acidic lumen, and this pH regulation has been shown to be tightly coupled with ionic (H^+, Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^-) flux/homeostasis of the lysosome. Moreover, recent studies have suggested that the ion fluxes may directly regulate lysosomal dynamics. $PI(3,5)P_2$ is an endolysosome-specific phosphoinositide that regulates ion homeostasis and membrane trafficking of endolysosomes via poorly understood mechanisms; human mutations of $PI(3,5)P_2$-metabolizing enzymes cause muscle and neurodegenerative diseases. Here we measure that sodium is the predominant cation in the lysosome using atomic absorption, indicating a large Na^+ concentration gradient is present across the lysosomal membrane. By the lysosome patch-clamp technique, we report that $PI(3,5)P_2$ specifically activates two novel Na^+-selective channels in the lysosome. Their identification provides insight into divalent cation selectivity, as well as to the mechanisms used by membranous lipids to directly regulate ion flux resulting in rapid changes in membrane potential and the fusogenic potential of intracellular organelles. We are currently investigating their physiological functions in detail.

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Type a Bax Channels: Electrophysiological Properties

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The pro-apoptotic protein, Bax, begins life as a soluble monomeric protein but can be activated to oligomerize and form two types of membrane channels: Type A channels are small and voltage-gated whereas Type B form larger channels and are voltage independent. To understand the properties that arise solely from the Bax protein and its interaction with a phospholipid membrane, Bax channels were formed and studied in planar phospholipid membranes. The Type A channels formed are oriented so that voltage-dependent closure occurs only at one sign of the membrane potential. An entire channel population responds in like manner but the sign of the voltage that results in voltage-gating differs from one experiment to another indicating that channel formation/insertion follows a form of auto-directed insertion as was first described for VDAC channels. Voltage-gating typically occurs at 70 mV resulting in a uniform population of 1.4 nS decrements. Three of these decrements appear to be associated with a single channel. Channel reopening/reassembly is a slow, multi-exponential process with the rate dependent on how long the channels were held closed. The use of triangular voltage waves reveals a complex behavior incompatible with the standard analysis applied to voltage-gated channels. These channels are unlikely to be responsible for protein release from mitochondria. Type B channels are likely to contribute to that process. The Type A channels may represent an alternative structure formed by activated Bax when cellular conditions do not quite warrant progression to the execution phase of apoptosis. (Supported by NSF grant: MCB-1023008).

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Potassium Channel in Mitochondria of Human Keratinocytes

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The outermost layer of human skin, epidermis, is predominantly composed of keratinocytes forming the barrier against the environmental damage of the skin caused by pathogens, UV irradiation, heat or water loss. It has been proved that UV irradiation or bacterial invasion induces apoptosis of keratinocytes via the mitochondrial pathways for caspase activation. Recent studies have shown that activation of mitochondrial ion channels protects mitochondria and cells from death by decreasing mitochondrial transmembrane potential.
The aim of this study was to identify inner membrane mitochondrial ion channels in human keratinocytes HaCaT cell line with patch-clamp technique. The observed channel has conductance equal to 80 pS at positive voltages. The I-V curve indicates that observed channel is inward rectifying. Additionally, our data suggest that the channel is voltage-independent. The channel identity was proved by RT-PCR and immunofluorescence methods. With using RT-PCR method mRNA transcripts for TASK-3 channel were found. Strong colocalization of the TASK-3 - specific immunopositivity and mitochondrion-specific labeling has been observed what proved the presence of TASK-3 channel in mitochondria of HaCaT cells.
Our studies confirmed the presence of mitochondrial potassium channel in mitochondria of keratinocytes. The channel is potential target for UV-induced skin damage. Its detailed pharmacology needs further investigations.
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Endoplasmic Reticulum & Protein Trafficking

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Electrostatic Regulation of CFTR Trafficking by the R Domain

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a member of ATP-binding cassette (ABC) superfamily. However, it functions as a chloride channel with a unique regulation (R) domain flanked by two intracellular nucleotide-binding domains (NBDs) and two membrane-spanning domains (MSDs). It has been reported that the R domain has multiple phosphorylation sites and regulates CFTR trafficking in response to cAMP/PKA stimulation. However, it is unclear which phosphorylation site is responsible for the regulated CFTR trafficking. In this study, phosphorylation of S768 is proposed to promote CFTR trafficking by enhancing an electrostatic attraction between K946 of intracellular cytoplasmic loop 3 (ICL3) and D835 of the R domain. First, the S768D mutant significantly increased the maximal channel current in the inside-out patches and the fraction of the matured CFTR (band C in Western blot) while K946D decreased both the maximal channel current and the band C fraction. Second, not only H950D/S768D and K946D/S768D but also K946D/H950D exhibited a weaker band C expression and a lower maximal CFTR current than S768D. More importantly, K946D/H950D/S768D greatly hampered the protein transfer to the cell membrane because no clear band C was observed and no macroscopic current was elicited by ATP, curcumin and PKA. On the other hand, the defective K946D/H950D/S768D trafficking was rescued by D835R or K978C. In contrast, H950R/S768R was transferred to the cell membrane but its trafficking was prevented by D835R. Thus, a complex electrostatic interaction between the R domain and ICL3 may be responsible for PKA-dependent CFTR trafficking.

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Kv2.1 Cell Surface Clusters are Insertion and Retrieval Platforms For Kv Channel Trafficking at the Plasma Membrane

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The Kv2.1 delayed-rectifier K+ channel regulates electrical activity in neurons and plays a non-conducting role in SNARE-mediated protein fusion in neuroendocrine cells. Kv2.1 is unusual among voltage-gated K+ channels in that it localizes to micron-sized clusters on the cell-surface of neurons and transfected HEK cells. Within these clusters Kv2.1 is non-conducting. Here we demonstrate that these surface structures are specialized platforms involved in